Attorney Docket No. 5051-425 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Dominique Robertson
Application Serial No.: 09/281,528
Filed: March 30, 1999
For: METHOD OF SUPPRESSSING GENE EXPRESSION IN PLANTS

Group Art Unit: 1633
Examiner: A. Mehta

Mail Stop Non-Fee Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration of Dominique Robertson, Ph.D.

Under 37 C.F.R. § 1.132

I, Dominique Robertson, do hereby declare and say as follows:

1. I am a named inventor on United States Application No. 09/281,528 ("the '528 application") and of the subject matter claimed therein.

2. I have a Ph.D. degree in Plant Cell Biology from Cornell University in Ithaca, NY. I am a Professor of Botany and Genetics in the Department of Botany at North Carolina State University. I have been conducting research in the area of geminivirus induced gene silencing for 13 years and have co-authored 4 publications related to the area of geminivirus-induced gene silencing and 6 publications related to the area of geminivirus-host interactions.

3. The investigations described below were carried out in my laboratory at North Carolina State University in Raleigh, North Carolina, USA, under my direction and supervision according to the protocols set forth in the '528 application. These studies demonstrate that the geminivirus silencing vector and DNA constructs that are the subject matter of the composition and method claims pending in the '528 application can comprise a variety of geminivirus genomes and a variety of heterologous DNA sequences comprising a fragment of a plant gene endogenous to a variety of plants and that silencing can be achieved at the pre- and post-transcriptional level. On the basis of these studies, it is my belief that the claims of the present invention could be practiced with any geminivirus genome, any heterologous DNA sequence and any plant.

4. **Geminivirus genomes studied**

Attached herewith as Exhibit A are excerpts from manuscripts and slides showing data produced from studies in which geminivirus silencing vectors as claimed in the present inv ntion and employing a geminivirus genome of tomato

golden mosaic virus (TGMV) and cabbage leaf curl virus (CbLCV, now officially referred to as CaLCuV) were produced and tested for their ability to silence expression of an endogenous plant gene upon introduction into a plant cell. A.1. shows the structure of TGMV-derived silencing vectors and the location and size of tested heterologous DNA fragments from a cDNA encoding a gene required for chlorophyll formation. A.2. demonstrates that slight variations in sequence betw en the target host gene and the heterologous DNA carried by the silencing vector can still elicit a strong silencing response. A.3. shows that a second, distantly related geminivirus with a conserved genome organization can also be used as a silencing vector. A.4. compares silencing of the tobacco *ChlI* (sulfur) gene encoding magnesium chelatase subunit I and the *Arabidopsis ChlI* (also known as chlorata 42) gene. The TGMV::su construct produces extensive silencing in *N. benthamiana* and the CbLCV::CH42 construct produces extensive loss of chlorophyll in *Arabidopsis*. A.5. shows a table comparing sequence similarity of geminiviruses and demonstrates that the TGMV and CbLCV are distantly related and that several economically important viruses, such as Sida Golden Mosaic Virus, which infects cotton, are intermediate between tomato golden mosaic virus and cabbage leaf curl virus.

5. **Heterologous DNA sequences studied**

Attached herewith as Exhibit B are excerpts from published and unpublished manuscripts showing data produced from studies in which geminivirus silencing vectors as claimed in the present invention and employing a variety of heterologous DNA sequences were produced and tested for their ability to silence expression of an endogenous plant gene upon introduction into a plant cell. B.1. is a summary f TGMV vectors that have been inoculated into *Nicotiana benthamiana*. Some genes upregulated by geminivirus infection were silenced to determine if they were necessary for viral infection. These included PCNA and 3 unknown genes, clone 9, 25, and 37, (B.2.) identified by PCR-based subtraction of infected and uninfected plants (Eagle and Robertson, unpublished). Putative orthologs of these genes were tested in *Arabidopsis* to determine if they were needed for CbLCV infection (B.4.). Also tested in *Arabidopsis* were genes identified in other labs as being necessary for silencing (B.4.). There is precedent in *C. elegans* for silencing a gene that is known to be required for silencing. These experiments verified results in *Arabidopsis* null mutants, that RdRp (host RNA dependent RNA polymerase) was required for geminivirus induced gene silencing (chapter 5, Muangsan Ph.D. thesis, submitted). The retinoblastoma related protein and proliferating cell nuclear antigen proteins (B.2.) are essential for plant growth and would be embryo lethals if mutated. Silencing mediated by TGMV carrying a 623 bp fragment of pre-transcribed CaMV 35S promoter sequence is seen first as cell autonomous loss of green fluorescent protein expression (B.2, Fig. 5) and then as a stronger, non-cell autonomous silencing. B.3. is a summary of CbLCV vectors that have been inoculated into *Arabidopsis*.

6. **Plant species studied**

Attached herewith as part of Exhibit A are excerpts from manuscripts and slides showing data produced from studies in which geminivirus silencing vectors as claimed in the present invention and employing a variety of heterologous DNA sequences were produced and tested for their ability to silence expression of an endogenous plant gene upon introduction into the cells of plants of different species. Figures 2 and 3 in section A.4. show that different plants (*Nicotiana*, a member of the Solanaceae family and *Arabidopsis*, a member of the Brassicaceae family) infected with different geminivirus vectors show a similar response, uniform loss of chlorophyll in new growth, when homologous, endogenous genes are targeted.

These data demonstrate that two different geminiviruses, 17 different transcribed heterologous DNA sequences, one promoter sequence, and species from two different plant families were successfully employed in the compositions and methods of the claimed invention.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dominique Robertson, Ph.D.

10/30/03
Date